PATENT

REMARKS

The amendments to paragraphs beginning on 17, 18, 19, 21, 27, and 37 were made to clarify the nomenclature used for the nucleic acid and amino acid sequences of the invention. All reference to MXR2 genes and proteins have been removed, since no sequences identified as such are present in the Specification or informal Sequence Listing addendum. In addition all reference to an MXR sequence has been changed to consistently reflect the MXR1 usage in the remainder of the Specification.

Claims 1-20 are pending in this application. Claims 1-3, 7-13, 17 and 18 have been amended. The amendments to claims 1-3, 7-13, 17 and 18 correct the format of the SEQ ID NO: assigned identifiers for sequences designated in these claims in accordance with 37 C.F.R. §§1.821(2)(d) and WIPO Standard ST.25.

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-6, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Claims by the current Amendment. The attached pages are captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."** As a convenience to the Examiner, a complete set of the Claims, as amended herein, is also attached to this Amendment as an Appendix entitled "**PENDING CLAIMS WITH ENTRY OF THE AMENDMENT.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Kenneth A. Weber
Reg. No. 31,677

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
KAW:dmw

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 21 of page 17 has been amended as follows:

MXR1 ~~MXR~~ polymorphic variants, alleles, and interspecies homologs that are substantially identical to MXR1 ~~and MXR2~~ can also be isolated using MXR1 ~~and MXR-2~~ nucleic acid probes, and oligonucleotides under stringent hybridization conditions, by screening libraries. Alternatively, expression libraries can be used to clone MXR1 ~~and MXR2~~ polymorphic variants, alleles, and interspecies homologs, by detecting homologs immunologically with antisera or purified antibodies made against MXR1 ~~or MXR2~~, which also recognize and selectively bind to the MXR1 ~~or MXR2~~ homolog.

Paragraph beginning at line 28 of page 17 has been amended as follows:

To make a cDNA library, one should choose a source that is rich in the *MXR1* ~~*MXR*~~ mRNA, e.g., human colon carcinoma cells. Placenta tissue or fetal brain or liver tissue. The mRNA is then made into cDNA using reverse transcriptase, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening, and cloning. Methods for making and screening cDNA libraries are well known (see, e.g., Gubler & Hoffman, *Gene* 25:263-269 (1983); Sambrook et al. *supra*; Ausubel et al., *supra*).

Paragraph beginning at line 3 of page 18 has been amended as follows:

An alternative method of isolating *MXR1* ~~*MXR*~~ nucleic acids and their homologs combines the use of synthetic oligonucleotide primers and amplification of an

RNA or DNA template (see US Patents 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis et al., eds 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of ABC proteins directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Degenerate oligonucleotides can be designed to amplify ABC homologs using the sequences provided herein. Restriction endonuclease sites can be incorporated into the primers. PCR or other in vitro amplification methods may be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of ABC protein encoding mRNA in physiological samples, for nucleic acid sequencing, or for other purposes. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Paragraph beginning at line 7 of page 19 has been amended as follows:

To obtain high level expression of a cloned gene or nucleic acid, such as those cDNAs encoding the ABC protein MXR1, one typically subclones *MXR1* ~~*MXR*~~ into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, e.g., in Sambrook *et al.* and Ausubel *et al.* Bacterial expression systems for expressing MXR1 are available in, e.g., *E. coli, Bacillus sp.,* and *Salmonella* (Palva *et al.*, *Gene* 22:229-235 (1983); Mosbach *et al.*, *Nature* 302:543-545 (1983). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

Paragraph beginning at line 3 of page 21 has been amended as follows:

Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of MXR1 ~~MXR~~ protein, which are then purified using standard techniques (*see, e.g.*, Colley *et al.*, *J. Biol. Chem.* 264:17619-17622 (1989); *Guide to Protein Purification*, in *Methods in Enzymology*, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (*see, e.g.*, Morrison, *J. Bact.* 132:349-351 (1977); Clark-Curtiss & Curtiss, *Methods in Enzymology* 101:347-362 (Wu *et al.*, eds, 1983).

Paragraph beginning at line 11 of page 21 has been amended as follows:

Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g.*, Sambrook *et al., supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing MXR1 ~~MXR~~ protein.

Paragraph beginning at line 16 of page 27 has been amended as follows:

Thus, in accordance with preferred embodiments of this invention, preferred antisense molecules include oligonucleotides and oligonucleotide analogs that are hybridizable with *MXR1* mRNA. This relationship is commonly denominated as "antisense." The oligonucleotides and oligonucleotide analogs are able to inhibit the function of the RNA, either its translation into protein, its translocation into the

cytoplasm, or any other activity necessary to its overall biological function. The failure of the messenger RNA to perform all or part of its function results in a reduction or complete inhibition of expression of MXR1 ~~MXR~~ polypeptides.

Paragraph beginning at line 21 of page 37 has been amended as follows:

cDNA libraries constructed with the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (GIBCO-BRL, Rockville MD) using mRNA from mitoxantrone resistant S1-M1-80 human colon carcinoma cells can be used to isolate the MXR1 ~~and MXR2~~ nucleic acids of the invention.

In the Claims:

Claims 1-3, 7-13, 17 and 18 have been amended as follows:

1. (Amended) An isolated ATP-binding cassette protein having the following properties:

i. conferring mitoxantrone resistance to a S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~; and

iii. having a molecular weight between about 70 kDa and about 75 kDa.

2. (Amended) The ATP-binding cassette protein of claim 1 wherein the protein has 95% identity to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

3. (Amended) A eukaryote cell genetically altered to overexpress an ATP-binding cassette protein having the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

7. (Amended) A DNA encoding a ATP-binding cassette protein wherein the protein is characterized by having the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins consisting of those depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

8. (Amended) The DNA of claim 7, wherein the encoded protein has 95% identity to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

9. (Amended) The DNA of claim 7, wherein the DNA encoding the protein has a sequence identical to that depicted in SEQ ID NO:1 or SEQ ID NO:3 ~~Seq. ID. No. 1 or No. 3~~.

10. (Amended) A process for over expressing ATP-binding cassette protein in a cell comprising a first step of either:

i. transforming the cell with an expression cassette which directs the expression of ATP-binding cassette protein; or,

ii. selecting a cell having an endogenous copy of the ATP-binding cassette protein, and transforming the cell with DNA which can serve as an enhancing element or as a second promoter where the insertion is upstream of the endogenous promoter operatively linked to the ATP-binding cassette protein and where the inserted DNA increases the basal expression levels of ATP-binding cassette protein; and a second step of,

culturing the transformed cell under conditions where the levels of ATP-binding cassette protein are increased above the basal levels of the non-transformed cells with the proviso that the ATP-binding cassette protein has the following properties:

a. confers mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

b. specifically binds to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

11. (Amended) The process of claim 10, wherein the ATP-binding cassette protein has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

12. (Amended) The process of claim 10, wherein the protein has the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

13. (Amended) A method of screening for inhibitors of cytotoxin resistance in cells comprising the steps of :

(a) culturing a cell genetically altered by the introduction of heterologous DNA which permits the overexpression an ATP-binding cassette protein where the protein has the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~;

(b) contacting the cell with a cytotoxin in an amount that permits cell survival due to the resistance conferred by the ATP-binding cassette protein;

(c) contacting the cell with a compound that inhibits the biological activity of the ATP-binding cassette protein;

(d) detecting the inhibition by measuring growth inhibition of the cells.

17. (Amended) A method of claim 13, wherein the ATP-binding cassette protein has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

18. (Amended) A binding protein which specifically binds to an ATP-binding cassette protein which has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4 ~~Seq. ID. No. 2 or ID. No. 4~~.

PENDING CLAIMS WITH ENTRY OF THE AMENDMENT

1. (Amended) An isolated ATP-binding cassette protein having the following properties:

i. conferring mitoxantrone resistance to a S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4; and

iii. having a molecular weight between about 70 kDa and about 75 kDa.

2. (Amended) The ATP-binding cassette protein of claim 1 wherein the protein has 95% identity to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

3. (Amended) A eukaryote cell genetically altered to overexpress an ATP-binding cassette protein having the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4.

4. (As filed) A cell of claim 3, wherein the cell is genetically altered by transformation of the cell with an exogenous DNA comprising an expression cassette encoding the ATP-binding cassette protein.

5. (As filed) A cell of claim 4, wherein the expression cassette comprises a heterologous promoter operatively linked to the DNA encoding the ATP-binding cassette protein.

6. (As filed) A cell of claim 3, wherein the cell has an endogenous copy of the ATP-binding cassette protein and the genetic alteration comprises insertion of DNA which can serve as an enhancing element or as a second promoter where the insertion is upstream of the endogenous promoter operatively linked to the ATP-binding cassette protein and where the inserted DNA increases the basal expression levels of ATP-binding cassette protein.

7. (Amended) A DNA encoding a ATP-binding cassette protein wherein the protein is characterized by having the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins consisting of those depicted in SEQ ID NO:2 or SEQ ID NO:4.

8. (Amended) The DNA of claim 7, wherein the encoded protein has 95% identity to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

9. (Amended) The DNA of claim 7, wherein the DNA encoding the protein has a sequence identical to that depicted in SEQ ID NO:1 or SEQ ID NO:3.

PATENT

10. (Amended) A process for over expressing ATP-binding cassette protein in a cell comprising a first step of either:

i. transforming the cell with an expression cassette which directs the expression of ATP-binding cassette protein; or,

ii. selecting a cell having an endogenous copy of the ATP-binding cassette protein, and transforming the cell with DNA which can serve as an enhancing element or as a second promoter where the insertion is upstream of the endogenous promoter operatively linked to the ATP-binding cassette protein and where the inserted DNA increases the basal expression levels of ATP-binding cassette protein; and a second step of,

culturing the transformed cell under conditions where the levels of ATP-binding cassette protein are increased above the basal levels of the non-transformed cells with the proviso that the ATP-binding cassette protein has the following properties:

a. confers mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

b. specifically binds to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4.

11. (Amended) The process of claim 10, wherein the ATP-binding cassette protein has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

12. (Amended) The process of claim 10, wherein the protein has the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

13. (Amended) A method of screening for inhibitors of cytotoxin resistance in cells comprising the steps of :

(a) culturing a cell genetically altered by the introduction of heterologous DNA which permits the overexpression an ATP-binding cassette protein where the protein has the following properties:

i. conferring mitoxantrone resistance on S1-M1-80 human colon carcinoma cells when expressed in the cells; and,

ii. specifically binding to polyclonal antibodies which specifically bind to a member of the group of proteins depicted in SEQ ID NO:2 or SEQ ID NO:4;

(b) contacting the cell with a cytotoxin in an amount that permits cell survival due to the resistance conferred by the ATP-binding cassette protein;

(c) contacting the cell with a compound that inhibits the biological activity of the ATP-binding cassette protein;

(d) detecting the inhibition by measuring growth inhibition of the cells.

14. (As filed) A method of claim 13, where the cytotoxin is mitoxantrone.

15. (As filed) A method of claim 13, where the cytotoxin is daunomycin.

16. (As filed) A method of claim 13, where the cell is a carcinoma cell.

17. (Amended) A method of claim 13, wherein the ATP-binding cassette protein has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

18. (Amended) A binding protein which specifically binds to an ATP-binding cassette protein which has 95% homology to the amino acids depicted in SEQ ID NO:2 or SEQ ID NO:4.

19. (As filed) A binding protein of claim 18, wherein the binding protein is an antibody.

20. (As filed) A binding protein of claim 18, wherein the binding protein is a monoclonal antibody.